Autoimmunity on alert: naturally occurring regulatory CD4+CD25+ T cells as part of the evolutionary compromise between a 'need' and a 'risk'

Michal Schwartz and Jonathan Kipnis

Autoimmunity, at least in the central nervous system (CNS), is not only an outcome of immune system malfunction, but is the body's own protective mechanism against destructive self-compounds. Likewise, the naturally occurring regulatory CD4+CD25+ T cells have a physiological function, and are not merely an evolutionary adaptation to suppress self-reactive T-cell clones that escaped deletion in the thymus. We postulate that the regulatory T (Tr) cells are the product of an evolutionary compromise between the need for autoimmunity on alert for tissue maintenance and the need to control autoimmunity to avoid autoimmune disease. In the event of an insult to the CNS, the balance between self-reactive (effector) T cells and Tr cells determines the time of onset, the intensity and the duration of the autoimmune response. This response might thus represent an adaptive mechanism, which is optimal for day-to-day maintenance, but insufficient in extreme cases of CNS damage or failure of regulation. Downregulation or upregulation of CD4+CD25+ Tr cells might be a way to achieve better protection from neurodegenerative conditions induced by self-destruction or avoid autoimmune inflammatory disease development, respectively.

Michal Schwartz*
Jonathan Kipnis
Dept of Neurobiology,
The Weizmann Institute of
Science, Rehovot, Israel.
*e-mail: michal.schwartz@
weizmann.ac.il

Studies in our laboratory have provided evidence that an immune response against self-compounds residing in damaged tissues of the central nervous system (CNS) confers protection against destructive self-compounds [1,2].

Our early studies of injury to myelinated CNS axons (optic nerve and spinal cord) showed that the insult is followed by an accumulation of T cells at the site of injury. Systemic injection of activated T cells after injury led to an increase in the number of accumulated T cells, regardless of their antigenic specificity [3]. However, only T cells that encounter their specific antigen at the lesion site are capable of ameliorating the consequences of the insult [1].

Axonal insult in the CNS is followed by both immediate degeneration and delayed (secondary) degeneration (an insult-induced outcome of the hostility of the nerve's extracellular environment) [4–6]. T cells specific to myelin-associated self-antigens or to peptides derived from them are able to partially counteract the secondary degeneration, thereby improving recovery [1,7–10].

Subsequent studies revealed that the beneficial effect of the autoimmune T cells was not merely the outcome of an experimental manipulation, but the result of a physiological response [11–13]. We showed that the ability to resist the consequences of CNS insults is T-cell dependent. Thus, rodents deprived of T cells showed worse recovery from both glutamate toxicity and axonal injury than matched wild-type controls. Moreover, splenocytes withdrawn from rats with injured spinal cords and passively transferred into naïve rats were found to be neuroprotective [11]. This beneficial T-cell-mediated response has been demonstrated as autoimmune in nature. Thus, the response to axonal injury in adult rats that were neonatally immunized (tolerized) to myelin-associated self-proteins was significantly worse than neonatally immunized with ovalbumin controls [2].

Additional studies showed diversity among strains in their ability to manifest an insult-evoked protective autoimmunity. Strains that are genetically resistant to induction of experimental autoimmune encephalomyelitis (EAE) on challenge with any myelin antigen are also relatively resistant to the consequences of CNS injury, and, as a corollary, strains that are susceptible to EAE induction are less resistant to injurious conditions [13]. Moreover, susceptible strains deprived of T cells or neonatally immunized with myelin did not differ in the recovery from their matched controls. This finding indicated that susceptible strains are deficient in their T cell-dependent protective mechanism [13].

Does protective autoimmunity apply only to insults to myelinated axons or is it more generally applicable?

To determine whether the observed protective autoimmunity is antigen-specific, it was examined whether myelin-specific T cells are neuroprotective against an insult inflicted at a site that is myelin-free. The chosen model was that of retinal ganglion cells directly exposed to toxic amounts of glutamate, a major player in neurodegenerative conditions. In the absence of T cells the ability to resist glutamate toxicity was reduced [13,14]. These neurons, however, unlike neurons affected by axonal injury, cannot benefit from vaccination with myelin antigens [15]. Vaccination with dominant self-antigens residing in the eye (inter-photoreceptor binding protein (IRBP) and S-Ag, known to be associated with uveitis, a common ocular autoimmune disease) was found to be neuroprotective [16]. These findings led us to suggest that for the T cells to display their neuroprotective effect they need to be activated, and that their local

activation demands specificity to antigens residing in the site. According to this view, the protective self-antigen and the antigen associated with autoimmune disease development in a particular tissue (although not necessarily their relevant epitopes) are identical and are specific to the site and not the type of lesion.

How can protective autoimmunity be reconciled with previous theories of self/nonself discrimination?

Immunologists differ in their views of the mechanisms needed to ensure nonresponsiveness to self-antigens. Our view, based on our experimental evidence suggesting that autoimmunity should be constantly on alert for immediate protective action, is that at least some self-reactive T cells are released to the periphery after their positive selection in the thymus for this protective purpose [2,10,13].

Elimination of self-reactive immune cells was first described with respect to B cells [17–22]. This theory was later also applied to T cells, when it was postulated that autoreactive T-cell clones are eliminated in the thymus [23,24]. Such deletion has been widely viewed as one of the mechanisms for avoiding autoimmunity, a response that until recently was understood mainly in terms of an attack by the body against itself. The assumption was that during development of T cells in the thymus, autoreactive clones that show high-affinity recognition of the self-peptide in the MHC groove will be eliminated. Bretscher and Cohn were the first to suggest that discrimination of self from nonself may not be the only mechanism for avoiding autoimmunity [25]. Janeway postulated that discrimination of self from nonself is based on the infectivity of the pathogen, and that the immune system discriminates self, which is noninfectious, from nonself, which is infectious [26]. Cohen, in his theory of the 'immunological homunculus', suggested that some autoreactive clones specific to dominant self-epitopes might be positively selected by the body for the purpose of regulation [27]. In 1994, Matzinger published her 'danger' theory [28], which postulates that any immune response is accompanied by an autoimmune response [29]. Later on, it was suggested that the thymus is responsible for positive (on thymic epithelium) and negative (on hemopoietic cells within the thymus) selection [30–32]. Modigliani et al. postulated that the factor determining whether thymic selection will be positive or negative is the avidity of the interaction between the antigen-presenting cells (APCs) residing in the thymus and the immature T cells [33]. For a given T-cell receptor (TCR) and MHC–peptide complex, the avidity of the interaction is defined as the product of the affinity of the TCR for the MHC–peptide complex and the number of copies of this complex expressed on the selecting cells [33]. Among the positively selected T cells, those with higher avidity would exit the thymus as activated cells, and that these T cells

would act as suppressors in the periphery. This led to the suggestion that an additional population of T cells (CD4$^+$) exits the thymus and serves a suppressive function in the periphery [34].

CD4$^+$ suppressor T cells were recently identified as naturally occurring T cells constitutively expressing CD25 markers [35,36]. It was suggested that the role of these thymus-derived regulatory T (Tr) cells is to ensure peripheral tolerance to potentially auto-aggressive T cells that escaped deletion in the thymus [37,38]. This suggestion was primarily based on the widely accepted notion that for the individual's best adaptation, autoimmune T cells should be maintained in a state of tolerance, anergy or general nonresponsiveness.

Tr cells as natural inhibitors of protective autoimmunity

Our data suggested that the preferential state of autoimmunity is not nonresponsiveness but the availability of autoimmune effector T cells that are suitably regulated and on alert for low-threshold activation by their relevant antigens. We found that animals deprived of Tr cells recovered better from CNS insults than their matched controls [36]. More direct proof of the suppressive effect of the Tr cells on spontaneously evoked T-cell-dependent protective immunity came from the finding that nude mice replenished with splenocytes from wild-type mice deprived of CD4$^+$CD25$^+$ T cells recovered better from CNS insult than either wild-type or nude mice replenished with a whole-splenocyte population. In addition, wild-type mice injected immediately after injury with CD4$^+$CD25$^+$ T cells recovered worse than wild-type mice that were not injected or which were injected with a population of CD4$^+$C25$^-$ T cells (i.e. effector cells) [2]. The general belief is that Tr cells act specifically on autoantigens and limit the autoaggressive response in space or time or both [39–41]. Therefore, it is possible that the elimination of Tr cells immediately after an injury allows rapid activation of the relevant autoimmune clones. The suppression induced by the Tr cells might be mediated through cytokines [transforming growth factor (TGF)-β or interleukin (IL)-10] or cell–cell contacts or both [42–46]. We suggest that the presence of Tr cells, regardless of their mechanism of action, represents a compromise between the need for autoimmune T cells and the need to avoid autoimmune disease. Thus, in contrast to common hypotheses in which naturally occurring Tr cells are perceived as a safety mechanism developed through evolution for the purpose of avoiding autoimmunity, we postulate that these T cells developed with the purpose of allowing the existence of well-regulated autoimmunity without the risk of autoimmune disease. We envisage a competition between two types of potential 'enemies' that emerge within the body in response to CNS insult, namely destructive self-compounds and autoimmune T cells of destructive (disease-causing) potential. The naturally
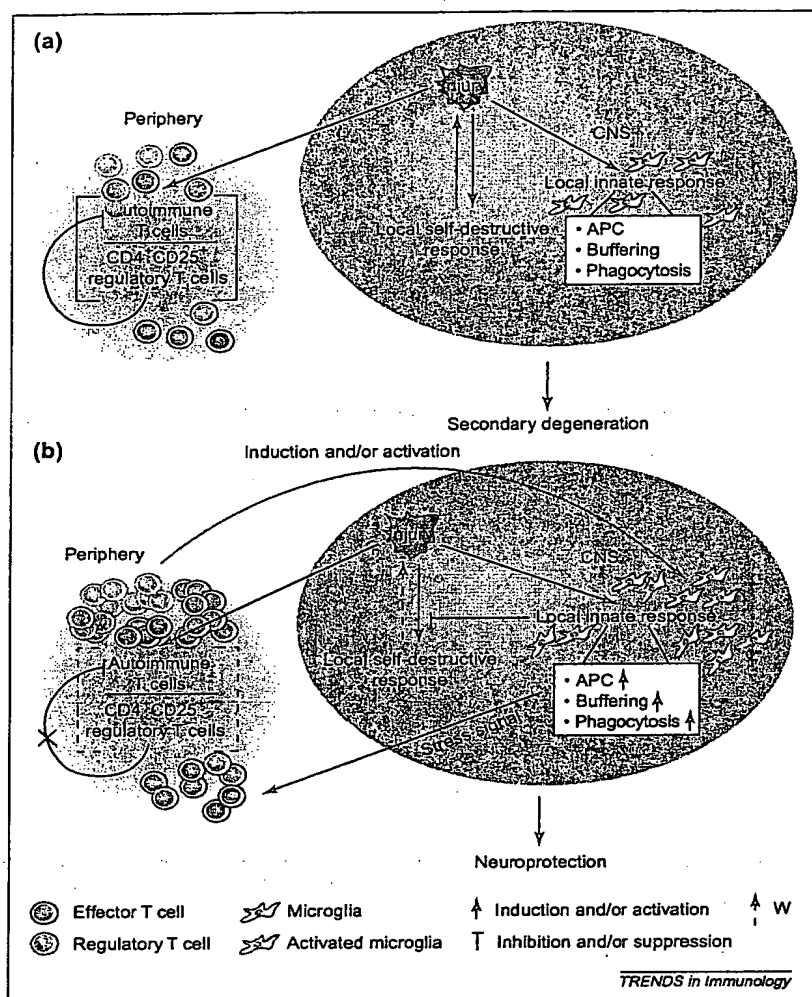
Fig. 1. Insult to the central nervous system (CNS) triggers a local self-destructive process and innate immune response that is beneficial in its purpose if well-controlled, but is too weak and needs to be amplified. CNS injury triggers local changes that cause secondary degeneration. Among the mediators of degeneration are destructive self-compounds, such as physiological substances (e.g. glutamate) present in toxic excess of their normal concentrations, and compounds associated with oxidative stress. In addition, the injury triggers a local innate response in which microglia are major players, capable of acting as antigen-presenting cells (APCs), phagocytic cells or neutralize significant amounts of toxic molecules [i.e. Glu, nitric oxide (NO)] [50,51]. The injury also activates an autoimmune T cell normally kept in the periphery in a suppressed state because of CD4⁺CD25⁺ regulatory T (Tr) cells (a). In the absence of a systemic autoimmune response, the local innate response is not strong enough to overcome the local destructive response, and the tissue consequently undergoes secondary degeneration. If the stress signal evoked by the injury is sufficient to overcome the suppression maintained by the CD4⁺CD25⁺ Tr cells, an autoimmune response will be activated. Autoimmune effector T cells will then home to the damaged CNS, where they will encounter their specific antigens presented on APCs, and will therefore be activated locally. The activated T cells will secrete cytokines and other soluble factors, thus amplifying and regulating the local innate immune response (b). Thus, a timely and efficient innate response will outweigh the local threat of the self-destructive response, attenuate secondary degeneration and protect neurons that escaped the initial injury.

the intensity, time of onset and duration of the autoimmune response (Fig. 1), which in turn determine whether it will fall within the therapeutic window of the particular insult. This might explain why transgenic mice that overexpress the TCR for myelin basic protein (T_{MBP}/Reg^+), having a relatively large number of Tr cells, recover better than the wild-type from optic nerve injury [11], but not from spinal cord injury [47]. The constitutive ratio of Tr cells (such as CD4⁺CD25⁺ T cells) to autoimmune effector T cells in the periphery depends on the affinity and abundance of the epitope that was presented on APCs in the thymus.

The probability of finding effector T cells with high affinity for a particular epitope in the periphery is inversely related to the abundance of that epitope on thymic APCs. According to this view, it is possible that in strains that lack the ability to spontaneously manifest a protective autoimmunity (and, as a corollary, are relatively susceptible to autoimmune disease development), the repertoire of antigens presented in the thymus differs from that in relatively resistant strains, and consists predominantly of epitopes characterized by high-affinity interaction with MHC class II molecules. Therefore, most of the T cells in the periphery of susceptible individuals will either be Tr cells (if not deleted), or high-affinity effector cells that have escaped deletion. Thus, under normal circumstances, the autoimmune effector T cells found in the periphery will be tightly regulated by the abundant Tr cells. As a result, when a protective autoimmune response is needed it will be evoked relatively late, and may not occur within the therapeutic window, at least if the insult is acute. Once the regulatory network has broken down (for example, as a result of immunization in which the inoculum is used in conjunction with a strong adjuvant), the autoimmune activity of the released effector T cells will be directed mainly to the same dominant epitopes, and therefore as well as leading to potential protection, will also probably lead to the development of an autoimmune disease. According to our hypothesis, and in line with the experimental evidence, such individuals will not only be prone to autoimmune disease, but will also possess only a limited ability to spontaneously manifest protective autoimmunity [13].

According to this perception, the two phenomena – the one traditionally viewed as peripheral tolerance to self and the other proposed here as the maintenance of self-reactive clones on alert for protective action – are both dependent on the same mechanism, and although they both relate to the goal of avoiding autoimmune disease, they differ with regard to the perception of autoimmunity that they reflect. From an evolutionary perspective, it seems that it would have been too great an investment if the sole function of the naturally occurring Tr cells had been to paralyze 'escapees' (autoimmune T cells that succeeded in escaping to the periphery).

occurring CD4⁺CD25⁺ Tr cells are the cells that maintain a balance and ensure that the competitor with potentially positive input will prevail. The presence of Tr cells, according to our view, is to allow 'differential activation' of some but not all of the autoimmune effector T cells. The ratio between the numbers of regulatory and effector cells determines

Protective autoimmunity - its role and mechanism

In our view, the function of autoimmunity is to augment the body's defense against the threat posed by its own destructive self-compounds. Thus, an important task of the anti-self T cells is to control local innate immune cells, such as the phagocytic cells that clear the damaged area of potentially destructive self-components, such as cell debris and other threatening matter [48]. This clearance service does not come free of charge. The potential damage because of harmful self-compounds is averted at the expense of the damage caused by the immune activity itself (U. Nevo *et al.*, unpublished). At an early stage of our work we suggested that this particular damage might be part of the mechanism of the repair itself, in that it contributes to the repair. Thus, for example, on observing that myelin basic protein (MBP)-specific autoimmune T-cell line cause a transient reduction in conductivity [1], we postulated that this transient reduction serves a useful purpose, as it has a hypothermia-like effect, which reduces energy consumption. Whether the cost is part of the mechanism, or is an inevitable negative side effect, remains to be resolved. Our current results suggest that autoimmunity implies the well-controlled ability to direct 'amplifiers' (autoimmune T cells) of the local innate response to the lesion site. It therefore seems that antigenic specificity determines the homing of T cells and their subsequent reactivation at the site of injury. Our data strongly suggest that Th1 cells are indeed necessary for protection [11]. Moreover IFN-γ, the cytokine that is most prominent in Th1 cells, activates the resident microglia in such a way that they become effective APCs, which further amplify the innate response (Shaked *et al.*, unpublished) [48]. At this stage it is not clear whether beneficial autoimmunity is applicable to other tissues or is manifested only in the brain and the eye. If the above scenario is an accurate reflection of reality, this would mean that the difference between 'good' and 'bad' autoimmunity lies not in the types of cells, but in their amounts and the duration of their activity (Fig. 1). Future studies should investigate the possibility that

this phenomenon applies to different insults (whether trauma- or microbe-induced) and to tissues other than neural tissue. For example, microbe-induced damage might not only trigger an immune response against the invaders, but could also activate a purposeful autoimmune response, similar to the induction of an autoimmune response by a traumatic injury [49], designed to cope with the consequences of the microbe-induced damage to the tissue.

Concluding remarks

According to our proposal, immunity and autoimmunity are two arms of the body's protective apparatus, making use of the same players, language (cytokines) and physiological devices (such as recognition of APCs). These two arms are thus similar in their technical operation but guided by different fundamental principles in terms of activation and regulation. In principle, the best form of adaptation for prevention of autoimmune disease would be deletion in the thymus of all self-reactive T cells. Alternatively, the best form of adaptation for coping with adverse conditions (such as CNS injury) would be the opposite – positive selection of all self-reactive T cells, including those directed to dominant epitopes, without any inhibitory peripheral regulatory network. We suggest that the evolutionary resolution, in the interests of optimal day-to-day maintenance of these two conflicting requirements, has resulted in deletion of effector T cells specific to extremely dominant epitopes in the thymus and, as an additional safely measure, the development of a naturally occurring regulatory mechanism. This optimal solution, however, is apparently insufficient in cases of trauma or other extreme situations that lead to ongoing degeneration requiring either upregulation of autoimmune effector T cells or downregulation of regulatory (suppressor) T cells. As a classic illustration of Darwin's theory of natural selection and survival of the fittest, it appears that avoiding risk (autoimmune disease) to healthy individuals is more important than helping injured individuals to survive.

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